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Phytochemical Screening and Toxicological Evaluation Using Brine Shrimp Lethality Test (BSLT) of Some Fractions of Prasman Leaves (*Eupatorium triplinerve* V) Extract

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Abstract

Prasman leaves (*Eupatororium triplinerve* V) was well documented to have anti cancer benefit in Indonesian traditional medicine history. However, there were no scientific studies including toxicological assessment on the plan extract. The purpose of this study was to investigate the toxicological effect of some fractions of Prasman leaves methanol extract. Phytochemical screening by the Farnsworth method on powder and some fraction of the methanol extract were conducted followed by toxicity test using the "Brine Shrimp Lethality" test (BSLT) method. In the current study results, the phytochemical screening showed the presence of flavonoid, saponin, coumarin, tannin, steroid and volatile oil. LC_{50} of the *n*-hexane fraction 238.66 µg/mL, ethyl acetate fraction 24.42 µg/mL, and *n*-butanol 64.10 µg/mL.

Keywords: BSLT, Toxicity test, *Eupatororium triplinerve*

INTRODUCTION

According to the World Health Organization database in 2003, there were ten million new cancer cases, with an annual increase of 20% every year. Based on this data and statistical calculation, it is estimated that in 2020, the new cancer cases may rise to as high as 20 million per year and around 84 million people could die if there were no comprehensive steps taken to address the problems (Anonim, 2009a).

Research on plants as one of the most potential source of chemotherapy treatment is currently on going. One of these supposedly beneficial plants is *prasman* leaves (*Eupatorium triplinerve* V) from the family of *asteraceace* and had been identified by the Indonesian society to use as diuretics, anticoagulant, anti-tumor agent; effectively relief nose bleed (epistaxis), high fever, cold and flu; stomatitis (aphtous ulcer) and as medicinal agents that help regulating menstrual cycle in woman, appetite booster and the formation of cicratic during healing process (Anonim, 2009a). Empirical consumption of this plant extrat however was based on remedial belief which passed on from one generation to the next without clear guidelines or dosage intake. This could be fatal to one's health as these natural ingredients may contain chemical substances that are highly toxic even in minute amount. This made standardization of traditional medicine as phytopharmacoproduct difficult. Remembering the diverse medicinal activity of Prasman leaves, scientist were encouraged to conduct studies on this plant extract to search for strong scientific evidence which could be useful as template for discovery of new anti cancer and ultimately Prasman Leaves can be used as alternative medicine in cancer treatment(Anonim, 2009b).

The current research study investigated the toxicity of fractions of prasman leaves methanol extract via phytochemical screening Farnsworth (1996) and the well known toxicity test Brine Shrimp Lethality (BSL) test.

BSLT was a well documented toxicity assessment method for analysis of bioactive substance from nature and ultimately lead to measurement of IC_{50} of toxic component of the plant extract. This test was carried out using the sea water, so called brine, containing shrimp larvae *Artemia Salina* Leach as media (Meyer et al., 1982).

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If the BSLT showed that the extract of plant of interest has potent level of toxicity, then further studies with strong focus on anti cancer drug development of this plant will be performed in more detail (Hendrawati, 2009).

METHODS

First of all, Prasman plant were collected and identified in The Herbarium Bogoriense in Bogor. They were washed thoroughly until clean using tap water, air dried at room temperature and ground to powder. Then, phytochemical screening Fransworth was performed on the crude powder and also for the extract after that followed by the BSLT.

Brine shrimp lethality (BSL) test:

Artificial seawater was prepared by mixing 38 g of salt with ZERO sodium content in 1 L distillated water (water with only 3.8% salinity content). The mixture was then filtered through the filter paper. A box with separator in the middle was made to become a box with two rooms. Filled the box with artificial sea water. The first side of the box was filled with artificial seawater and 50-100 mg of the shrimp eggs for hatching, then covered with the aluminium foil. Into the side two of the box, light source of TL18 watt was placed and exposed to the light source for 24hr. These eggs hatched to shrimp larvae. Successfully hatched larvae were selected and pick up by pippette for use in the test.

After that concentrated mixture of the test solution was prepared by dissolving 50 mg of the concentrated extract in 5 ml of DMSO. This was then diluted into diluted test mixture at concentrations of 10 – 1000 □g/ml. in vials.

Toxicity test using Meyer method (Fransworth, 1996): Base solutions containing fractions of the prasman leaves extract (5, 10 and 50 □l) and its crude powder extract were added accurately via pipette into vials to give solution concentration of 10, 100, 1000 □g/ml. They were completely evaporated and were prepared in triplicate. Then, 2 ml of artificial seawater was added to each test sample vial with the aid of DMSO (1%) if solubility problem arised when making test solution. Finally, ten (10) *naplius artemia salina* Leach shrimp larvae was added followed by the addition of more artificial seawater to make up to a total volume of 5 ml.

Control solution was prepared in the same way as the test solution in the absence of prasman leaves extracts and fractions. Then, artificial seawater and larvae was added and solution was made to total volume of 5 ml. After 24 hr incubation, the number of mortality of the shrimp larvae was counted.

RESULTS

The phytochemical screening via Farnsworth method was conducted using crudecpowder and fractions of prasman leave extract. In crude powder of prasman leaves, presence saponin, tannin, steroid,coumarin and volatile oil. The results of Phytochemical test is shown in Table I.

DISCUSSION

Our study results showed that the phytochemical screening of prasman leaf crude powder, the presence of essential oil compounds and steroids in n-hexane extract were observed. Flavonoid were found in the EtOAc and n-BuOH extracts, due to their solublity in polar and semipolar solvents. Interestingly, there were two types of Flavonoids, they are flavonoid that is linked to sugar moiety hencedissolve in polar solvents, and those with non-sugar moiety linkage hence it will not be soluble in semipolar solvents. Saponins and tannins were found in n-BuOH extract, moreover, coumarins were present in the EtoAc extract. Some classes of these compounds have pharmacological activities including anticancer, this statement in line with Kurz and Constabel (1998). Overall, the presence of these bioactive substances also confirmed the traditional use of prasman leaves to treat anti cancer by the layman in the Indonesian society. This lead to the possibility for prasman leaves to there was become future potent anti cancer drug.

As shown in the result table II, there was a correlation between the amounts of extractive substances with the mortality rate of the tested *Artemia salina* larvae shrimp, the increased tendency of the larvae mortality rate is in line with the increased dose of the extractive substance concentration.

The result of each organic solvent partitioning revealed a different effect on the mortality rate of the tested *artemia salina* larvae shrimp. This was caused by the different extractive substance content in each solvent.Toxicity evaluation via BSLT method required high precision, because many factors can affect the mortality of larvae shrimp *Artemia salina* L. The shrimp larvae are very sensitive to any substance presence within their habits. Their skin is a thin membrane circumstances which allow diffusion of substances from environment, affecting their

metabolism. In addition to their sensitivity to the environment. *Artemia salina* L grow so rapidly that it resembled the growth rate of cancer cell.

Table I. Results of phytochemical screening of Prasman powder and extract

Chemical content	Powder	*n*-hexane Extract	Ethyl Acetate Extract	*n*-buthanol Extract	Water extract
Alkaloid	-	-	-	-	-
Flavonoid	+	-	+	+	-
Saponin	+	-	-	+	+
Tannin	+	-	-	+	+
Quinon	-	-	-	-	-
Steroid/	+	+	-	-	-
Triterpenoid	**Steroid**	**Steroid**			
Coumarin	+	-	+	-	-
Volatile oil	+	+	-	-	-

+ positive reaction - negative reaction

The addition of extractive substance from the *prasman* leaves that may contain an active substance has disrupted the larvae metabolism resulting in its death. The demise of the larvae shrimp is also presumed to be the death of the cancer cell. Meanwhile the death of the larvae shrimp in the control batch is caused by factors beyond control such as temperature, humidity, light intensity and the lack of other food sources (Juliasman, 2006).

BSLT test result demonstrated strong correlation with screening tests result of the prasman leaf. The phytochemical screening clearly indicated the presence of flavonoids, saponins, coumarins, tannins, steroids and volatile oil. These bioactive compounds were isolated and re-dissolved in EtoAc. Flavonoid was, suspected to cause the death of larval shrimp *Artemia salina* L. The n-butanol soluble bioactive compounds, flavonoids, saponins, and tannins, form the nature of synergism. Meanwhile, the bioactive compound which dissolved in *n*-hexane solvent and causes the death of the *artemia salina* larvae shrimps is classified as a steroid. Based on Vickery and Bickery (1991) statement, steroid and saponin in plants will act as poison for insects, bacteria and fungi, and could be used as a medicine to prohibit the growth of tumor cells in plants and animals.

Further studies is required to isolate and identify the active components of ethyl acetate fraction that has cytotoxic effect and to conduct a pharmacological activity test to prove its activity before we can get its benefit as an alternative cancer treatment.

Table II. LC_{50} with BSLT Method

Sample	Concentrration µg/mL	Number of death cell	Number of viable cell	Acumulation of death cell	Acumulation of viable cell	Acumulation death/total cell	% mortility cell	LC_{50}
N –hexane	1000	17	13	33	13	33/46	71.73	
	100	10	20	16	33	16/49	32.65	238.66 µg/mL
	10	6	24	6	57	6/63	9.52	
Ethyl acetate	1000	30	0	63	0	63/63	100	
	100	18	12	33	12	33/45	73.33	24.42 µg/mL
	10	15	15	15	27	15/42	35.71	
N-Buthanol	1000	28	2	52	2	52/54	96.29	
	100	17	13	24	15	24/39	61.35	64.10 µg/mL
	10	7	23	7	38	7/45	15.55	

Water	1000	4	26	7	25	7/33	21.21	
	100	2	28	3	54	3/57	5.26	1184130.90 µg/mL
	10	1	29	1	83	1/84	1.19	

CONCLUSION

The phytochemical screening showed the presence of flavonoid, saponin, coumarin, tannin, steroid and volatile oil. The result of the toxicity test using BSLT method, showed all of the fraction of methanol extract have toxicity activity (LC_{50} < 1000 µg/mL) Water extract have no toxicity activity. The higest LC_{50} for ethyl acetate (24.42 µg/mL) and the lowest LC_{50} for n hexane (238.66 µg/mL).

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